COMPARISON OF ^{3}H-NICOTINE AND ^{3}H-PEMPIDINE BINDING *IN SITU* TO RAT BRAIN SLICES.

Thomas J. Martin and Billy R. Martin

Department of Pharmacology and Toxicology, Medical College of Virginia/ Virginia Commonwealth University, Richmond, Virginia, USA

Initially, evidence for the existence of central nicotinic receptors was based on the fact that nicotine exerted a myriad of central effects that were antagonized by ganglionic blockers capable of penetrating the blood-brain barrier, such as mecamylamine and pempidine. It is interesting that none of these antagonists displace ^{3}H-nicotine binding *in vitro*, suggesting that nicotinic agonists and antagonists have distinct binding sites. ^{3}H-Nicotine and ^{3}H-pempidine binding was studied *in situ* to 20 µm slide-mounted rat brain slices. The sections were incubated in various concentrations of ^{3}H-ligand for 2 hrs. in 50 mM phosphate buffered saline, pH=7.4 on ice, followed by four consecutive 30 sec. rinses in 500 ml of ice-cold buffer. The sections were then wiped from the slides with filter paper, solubilized, and counted by scintillation spectroscopy. Nonspecific binding was assessed in the presence of 10^{-4} M unlabelled ligand. ^{3}H-Nicotine binding displayed high- and low-affinity binding sites with Kd's and Bmax's of 2 nM and 67 fmol/mg protein, and 99 nM and 482 fmol/mg protein, respectively. ^{3}H-Nicotine binding at 1 nM was displaced by (-)cytisine, (-)nicotine, (+)nicotine, and (±)anabasine with IC_{50}'s of 0.61, 1.54, 62.3 and 140.9 nM, respectively. Neither mecamylamine nor pempidine displaced this binding at concentrations up to 10^{-3} M. ^{3}H-Pempidine binding under these conditions was neither saturable nor displaceable. It has been suggested that these antagonists bind to the open-channel form of the receptor-ionophore complex only. Addition of nicotine to the incubation media resulted in a slight stimulation of ^{3}H-pempidine binding. This stimulation was concentration-dependent at 4°C, but not at 25°C. It is clear that these antagonists do not bind to the nicotine binding site under conditions maximized for agonist binding. Supported by CTR grant # 2130.

Keywords: nicotine receptor mecamylamine pempidine *in situ* binding displacement

NORADRENALINE SYNTHESIS INDUCED BY SYSTEMIC NICOTINE ADMINISTRATION: A REGIONAL STUDY IN THE HIPPOCAMPUS.

Stephen Mitchell, Michael Brazell, Markus Schugens and Jeffrey Gray.

MRC Brain, Behaviour and Psychiatry Research Group, Department of Psychology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London, SE5 8AF, U.K.

Previous in vitro biochemical studies using slice or synaptosomal preparations have indicated a sensitivity of rat hippocampal noradrenergic nerve terminals to relatively high concentrations of nicotine (NIC) (Balfour, 1973; Arqueros, 1978). We have recently shown that acute systemic administration of NIC increased dihydroxyphenylalanine (DOPA) accumulation following inhibition of amino acid decarboxylase with m-hydroxybenzylhydrazine (NSD-1015) by direct stimulation of central nicotinic receptors, (Mitchell et al., 1987). Furthermore, the induced response was inhibited by lesions of the ascending dorsal noradrenergic bundle, indicating that this reflected an increase in noradrenaline (NA) synthesis. We now extend these findings by investigating whether the induced response is localised within particular areas of the hippocampus: CA_1, CA_{3-4} and dentate gyrus (DG).

Male Sprague-Dawley rats (280 - 300g, Harlan Olac) received NIC (0.8 mg/kg, free base, s.c.) or saline (1 ml/kg) 15 min before NSD-1015 (100 mg/kg, i.p.). Rats were killed 30 min later, hippocampus dissected and cut into 450 µm slices (Sorvall TC-2) soaked in ice cold Krebs' solution and subdivided into CA_1, CA_{3-4} and DG. Samples were stored at -70°C, prepared by sonication and analysed by HPLC with electrochemical detection.

Although NIC significantly increased DOPA accumulation in all three areas, the induced response was significantly higher in the DG compared with CA_{3-4} and CA_1 ($P<0.005$ and $P<0.001$ respectively; Students' t-test). In all areas, 5-HTP accumulation (an index of 5-HT synthesis) was reduced by 19 - 20% (not significant). The difference in sensitivity of noradrenergic nerve terminals between the three areas may reflect a non-uniform distribution of nicotinic receptors.

Key Words: nicotine, hippocampus, dentate gyrus, noradrenaline synthesis

References

Arqueros, L., Naquira, D. and Zunino, E. (1978). Nicotine-induced release of catecholamines from rat hippocampus and striatum. Biochem. Pharmacol., 27, 2667-2674.

Balfour, D.J.K. (1973). Effects of nicotine on the uptake and retention of ^{14}C-noradrenaline and ^{14}C-5-hydroxytryptamine by rat brain homogenates. Eur. J. Pharmacol., 23, 19-26.

Mitchell, S.N., Brazell, M.P., Joseph, M.H. and Gray, J.A. (1987). Regional differences in the effect of nicotine on catecholamine turnover in the rat CNS. Br. J. Pharmacol., 91, 430P.

EFFECTS OF HYPOXIA ON NICOTINIC AND MUSCARINIC CHOLINERGIC RECEPTORS IN THE RAT BRAIN

Haruaki Ninomiya, Takashi Taniguchi and Motohatsu Fujiwara

Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

The effects of in vivo hypoxia (10 % O_2 , 90 % N_2) on the cholinergic receptors in the rat brain were examined using ligand binding techniques. Male Wistar rats were exposed to a mixture of 10 % O_2 and 90 % N_2 in a chamber for various periods (3, 6, 12 and 24 hours). The control rats were exposed to room air. The brain regions examined were the cerebral cortex, striatum, globus pallidus, hippocampus, thalamus and cerebellum. Nicotinic and muscarinic cholinergic receptors were assessed by ^{3}H-nicotine and ^{3}H-quinuclidinyl benzilate (QNB), respectively. Compared with the control values, the number of ^{3}H-nicotine binding sites was significantly reduced in number in the thalamus and hippocampus after 3-, 6- and 12-hour exposure to hypoxia. Scatchard analysis revealed that the decrease was due to a decrease in Bmax value of the high affinity binding sites. On the other hand, the number of ^{3}H-QNB binding sites was significantly increased in the globus pallidus after a 6-hour exposure to hypoxia and in the hippocampus after 6- and 12-hour exposure. In addition, carbachol-stimulated phoshoinositide turnover was increased in the hippocampus after a 6-hour exposure. Both the changes in nicotinic and muscarinic cholinergic receptors seemed to be normalized by 24 hours. These findings suggest that there are significant changes in nicotinic and muscarinic cholinergic receptors in the selected regions of rat brain under hypoxia.

key words: hypoxia - rat brain - nicotinic receptors - muscarinic receptors

ACUTE AND SUBACUTE EFFECTS OF NICOTINE IN A MOUSE MODEL OF ANXIETY: COMPARISON WITH THE REFERENCE ANXIOLYTIC DIAZEPAM

Emmanuel Onaivi, John Rosecrans and Billy Martin

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA, USA 23298

The behavioral effects of nicotine are associated with receptor mediated events and involve interaction with different receptors and neuronal systems while benzodiazepine are known to exert their anxiolytic effects by acting at the GABA/benzodiazepine receptor complex. Some antagonists that interfere with this receptor complex are known to counteract the anxiolytic effects of benzodiazepines in animal models of anxiety (see Treit, 1987). However, the neuronal processes involved with the behavioral actions of nicotine are not clearly understood.

In this study we describe the acute effects of nicotine and the reference anxiolytic diazepam in male ICR mice using the elevated plus-maze (Lister 1987). Nicotine (0.1-1.0 mg/kg, s.c., administered 10 min prior to testing) and diazepam (0.005-0.1 mg/kg, i.p., administered 30 min prior to testing) significantly increased the time spent and number of entries into the open arms of the maze during a 5 min test session which was considered to be a measure of anxiety. Pretreatment with flumazepil (10 & 20 mg/kg) reversed the anxiolytic effects of diazepam (0.01 & 0.05 mg/kg) but not that of nicotine (0.1 & 0.5 mg/kg). In contrast mecamylamine (0.5 & 1.0 mg/kg) did not alter the effects of diazepam but antagonized the effects of nicotine without precipitating an anxiogenic response.

In order to assess the development of tolerance to repeated administrations of these drugs, animals received daily injections of either nicotine (0.1 mg/kg) or diazepam (0.1 mg/kg) twice daily for 14 days and the abstinence withdrawal anxiogenesis upon abrupt cessation from treatment was measured. The daily administration of nicotine or diazepam led to the development of tolerance in the measures of anxiety responding. At 24 hr and 48 hr following withdrawal from the 14-day treatment schedule, an intense withdrawal anxiogenesis characterized by a significant increase and decrease in the time spent in the closed and open arms, respectively, was recorded for diazepam- but not nicotine-treated animals whose responses were indistinguishable from controls.

It is concluded that whilst the abstinence withdrawal anxiogenesis following abrupt withdrawal from diazepam may be linked to the development of tolerance, this cannot be presently discounted for the effects of nicotine as other neurochemical substrates may contribute to nicotine abstinence, and indeed two forms of tolerance to the effects of nicotine have been recognized in rats (Clarke 1987). Therefore the specificity of drug action on the neuronal nicotinic receptors and the value of the anxiety model to characterize the specificity of agonist/antagonist activity at the central nicotinic cholinoceptor sites remains to be determined.

Supported by NIDA grant DA-00490.

Key workds: Anxiety, nicotine, diazepam, elevated plus-maze, tolerance, mouse

PREDICTIVE RELATION OF HUMAN SMOKING BEHAVIORS TO THE PHARMACOKINETICS OF NICOTINE DERIVED FROM CIGARETTE SMOKE.

J. H. Robinson, J. D. deBethizy, D. W. Griffith, R. A. Davis, J. H. Reynolds, G. T. Burger and A. W. Hayes.

Biochemical/ Biobehavioral R&D, R.J. Reynolds Tobacco Company, Bowman Gray Technical Center, Winston-Salem, NC 27102, USA.

Human smoking behaviors were monitored in a study of the pharmacokinetics of nicotine (NIC) derived from cigarette smoke. Twelve male smokers were tested on 3 occasions, once smoking tobacco-burning cigarettes (Reference), once smoking cigarettes that heat, but do not burn tobacco (Test), and again smoking the Test cigarettes after smoking these cigarettes exclusively for 39 days. All subjects abstained from tobacco product use for 3 days prior to each smoking test. During each test, subjects smoked a total of 7 cigarettes, spaced at 30 min intervals. Puffing patterns were monitored for all cigarettes, breathing patterns were monitored during the smoking of the 7th cigarette, and venous blood samples were obtained for determination of plasma NIC and cotinine. Absorbed NIC was calculated by comparing area under the plasma curve (AUC) from the smoking studies to the AUC from an IV infusion. Mainstream smoke yields of NIC, carbon monoxide and carbon dioxide generated by the human smoking patterns were determined using a programmable smoking machine that reproduces the human smoking profile and captures the mainstream smoke. Average mainstream smoke yields of NIC ranged from 0.22 - 2.34 mg/cigarette and average NIC absorbed ranged from 1.8% to essentially 100% of the NIC present in the mainstream smoke. Significant smoking behavior correlates of mainstream smoke component yields and absorbed NIC were seen for both cigarettes. These results emphasize the importance of monitoring individual smoking behaviors in studies of the pharmacokinetics of NIC derived from cigarette smoke.

Key Words: Human Smoking Behavior, Nicotine, Pharmacokinetics, Cigarette Smoke

USE OF NEURONAL BUNGAROTOXIN TO STUDY NICOTINIC RECEPTORS IN RAT BRAIN

David W. Schulz and Richard E. Zigmond

Dept. of Pharmacology, Harvard Medical School, Boston, MA. 02115.

Neuronal nicotinic receptors in autonomic ganglia have been extensively characterized using neuronal bungarotoxin (NBT), a neurotoxin that is present along with alpha-bungarotoxin (BGT) in the venom of Bungarus multicinctus (Loring and Zigmond, TINS, 1988). We have investigated the possibility that this toxin may also block nicotinic synaptic transmission in the brain, and therefore may be a useful probe for studying nicotinic receptors in mammalian CNS tissue.

Nicotine is known to stimulate the release of dopamine from dopaminergic nerve terminals in the striatum. We have found that 100 uM nicotine increases the basal release of dopamine by at least 2-fold. This enhanced release is both calcium-dependent and antagonized by hexamethonium, suggesting that it is mediated by nicotinic receptors. NBT (100 nM) completely blocked this effect of nicotine, while BGT at the same concentration had no effect.

The binding of ^{125}I-NBT was studied using membranes prepared from various rat brain regions. The affinity for binding in the striatum was ca. 3 nM, with a B_{max} of 25 fmol/mg protein. Two types of NBT binding sites have been found in autonomic ganglia. One of these is also recognized by BGT, while the other is selective for NBT. In all areas of the rat brain examined, <30% of the high affinity ^{125}I-NBT sites were recognized by BGT. Of the NBT-selective sites, other nicotinic ligands such as nicotine and d-tubocurare competed for about 50% of binding. The distribution of ^{125}I-NBT binding in membranes from six brain regions was surprisingly homogeneous, and was not correlated with the distribution of ^{125}I-BGT binding.

In conclusion, we have shown that NBT is a functional antagonist of at least some nicotinic receptors in rat brain. While specific NBT binding sites exist in brain tissue, it appears that not all of these high-affinity sites are nicotinic in nature. Future experiments will focus on the autoradiographic localization of ^{125}I-NBT binding sites and further pharmacological characterizations of central nicotinic receptors.

Key words: neuronal bungarotoxin (NBT), alpha-bungarotoxin (BGT), nicotine, dopamine release, caudate-putamen, CNS nicotinic receptors.

NICOTINE METABOLISM IN NAIVE AND NICOTINE TREATED RATS.

D.W. Sepkovic, J.P. Richie, Jr., C.M. Axelrad, M.A. DeNicola, and N.J. Haley. Section of Clinical Biochemistry, Division of Nutrition and Endocrinology, American Health Foundation, Valhalla, N.Y. 10595 U.S.A.

The pharmacological effects of nicotine can be regulated by the rate of nicotine metabolism as well as tolerance to this alkaloid. Studies have suggested that induction of tolerance can affect metabolic rate and elimination of terminal metabolites. We investigated the development of nicotine tolerance on cotinine elimination in Fisher-344 male rats and inter-strain differences in nicotine metabolism in both naive and nicotine treated animals.

Nicotine was intravenously injected (1.0 mg/kg) into Fisher rats and urine was collected hourly for 48 hrs. Substantial concentrations of cotinine were observed after 1 hr. Maximum levels were reached within 6 hrs. and thereafter a monoexponential decline was observed. The mean biological half elimination time ($t1/2\beta$) was 6.7 ± 0.5 hrs. The same rats were then administered nicotine in drinking water (0.01%, w/v). At the end of each week, urine voids were collected, excretion rates and elimination t1/2 of cotinine were determined as the animals became acclimatized to nicotine. Additionally, 5 different strains of rats (n=30 per group) were administered nicotine in their drinking water. Each week, 5 animals per group were sacrificed and hepatic cytochrome P450 activities were determined. Baseline levels were similar in all strains (0.78-0.87 nmoles/mg microsomal protein) with the exception of the Brown Norway rat (0.58 nmoles/mg). P450 levels increased 16-78% in all species after 2 weeks of nicotine treatment and remained elevated through the 4 weeks of the experiment. The greatest induction occurred in the Brown Norway rat and the lowest in the Wistar rat. The levels and inducibility of P450 were then compared with the *in vitro* capacity for nicotine metabolism in the same liver homogenates.

Supported by NCI Grant PO1 CA29[illegible]

Key words: dependence, cotinine, cytochrome P450, enzyme induction

DIFFERENTIAL INHIBITION BY α-BUNGAROTOXIN AND d-TUBOCURARINE OF THE ^{125}I-α-BUNGAROTOXIN BINDING TO THE MEMBRANE FRACTION OF RAT CEREBRAL CORTEX.

Shoei-Yn Lin-Shiau

Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China.

The specific bindings of ^{125}I-α-bungarotoxin (^{125}I-α-BuTX) to the membrane fraction of rat cerebral cortex were characterized in the Krebs solution. Both unlabelled α-bungarotoxin (α-BuTX) and d-tubocurarine inhibited but atropine and hexamethonium did not inhibit ^{125}I-α-BuTX binding. This specific binding of ^{125}I-α-BuTX was dramatically increased by incubation in plain 0.32 M sucrose from a control maximal binding of 83.6±1.2 pmol/g protein to 1,196.1±27.5 pmol/g protein. Unlabelled α-BuTX inhibited ^{125}I-α-BuTX binding in 0.32 M sucrose as well as that in Krebs solution, but d-tubocurarine did not inhibit the binding in 0.32 M sucrose. Addition of various cations (2.5 mM Ca^{2+}, 20 mM Mg^{2+}, 150 mM NaCl or 150 mM KCl to various concentrations of sucrose in order to make isosmotic solution) not only decreased the total binding to the level similar to that in the Krebs solution but also recovered the inhibitory action of d-tubocurarine on ^{125}I-α-BuTX bidning. The tissue specificity of this sucrose effect was investigated and a similar effect was found in the cerebellum and leg skeletal muscle but not in the heart. These findings suggest that the conformation and abundance of binding sites for ^{125}I-α-BuTX in various tissues are markedly modulated by cations.

PRESYNAPTIC NICOTINIC AUTORECEPTORS IN THE HIPPOCAMPUS

B.Thorne[1], G.G.Lunt[1], S. Wonnacott[1] & T.C.Gallagher[2]

Department of Biochemistry[1] and Department of Chemistry[2], University of Bath, Bath BA2 7AY, U.K.

Postmortem biochemical studies of Alzheimer brain have shown a reduction in choline acetyltransferase activity, consistent with a loss of cholinergic terminals, primarily in the cortex and hippocampus. A decrement in the number of nicotinic acetylcholine receptors (nAChR) defined by [^{3}H]nicotine binding, has also been reported, whereas muscarinic receptors are little changed. This suggests a predominatly presynaptic localisation of nAChR. We have investigated the role of presynaptic nAChR in the regulation of acetylcholine (ACh) release in rat hippocampus. Initially synaptosomes were isolated by conventional sucrose density gradient centrifugation. Following uptake of [^{3}H]choline, nicotine-evoked release of [^{3}H]ACh from superfused synaptosomes was demonstrable, but rather low and difficult to quantitate. Nevertheless, the nicotinic nature of this response was established by its sensitivity to the antagonist dihydroßerythroidine. To improve the signal to noise ratio, highly purified synaptosomes were prepared by isotonic Percoll density gradient centrifugation (Dunkley et al., Brain Res. in press). Both [^{3}H]choline uptake (7.3pmol/min/mg protein) and [^{3}H]nicotine binding (400 fmol/mg protein) were maximal in fraction 4 on the gradient. These synaptosomes showed a 5-9 fold enhancement of nicotine-evoked [^{3}H]ACh release per mg protein, compared with earlier preparations. Nicotine-evoked release was Ca^{++} dependent. It is proposed that the presynaptic nAChR identified by [^{3}H]nicotine binding, modulates ACh release in the rat hippocampus. (Supported by Tobacco Advisory Council and an SERC CASE Studentship with Beechams Pharmaceuticals to B.T.).

Keywords: PRESYNAPTIC AUTORECEPTOR, HIPPOCAMPUS, SYNAPTOSOMES, [^{3}H]NICOTINE BINDING, ACh RELEASE.

EFFECTS OF PHENCYCLIDINE ON NICOTINIC RECEPTOR OF MOUSE DIAPHRAGM AND GLUTAMATE RECEPTOR OF DROSOPHILA MELANOGASTER

Ming-Cheng Tsai, Mai-Lei Chen and Chih-Chia Lai

Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.

Phencyclidine(PCP) abuse has become a major public health problem in the United States. The effects PCP on the post-tetanic potentiation(PTP) of synaptic transmission were studied on the isolated phrenic nerve diaphragm of mouse and the ventral lateral longitudinal fibre of the third instar lavae of Drosophila melanogaster. PCP potentiated the directly elicited muscle twitch while blocked the indirectly elicited muscle twitch. It also decreased the PTP of twitch tension and endplate potential(EPP) while it had no effect on the quantal content of EPP. The inhibitory effect of PCP on the PTP of twitch tension was dependent on the frequency and duration of tetanic stimulation. 4-Aminopyridine(4-AP) increased both directly and indirectly elicited twitch tension. However, 4-AP did not inhibit the PTP of the twitch tension. It seems that the voltage and time dependent effect of PCP on the endplate channel may contribute to its effect on PTP. L-Glutamate is an agonist of the excitatory transmitter at Drosophila larval neuromuscular junction. PCP also decreased the excitatory junction potential of the third instar larvae. It is suggested that the inhibitory effects of PCP on the glutamate and nicotinic receptors may contribute to the clinical symptomatology induced by PCP. Supported by National Science Council, NSC-77-0412-B002-69, R.O.C.

PHENCYCLIDINE, SYNAPTIC TRANSMISSION, POST-TETANIC POTENTIATION, DRUG ABUSE, FRUIT FLY, NICOTINIC RECEPTOR, GLUTAMATE RECEPTOR.

Up-regulation of nicotinic receptors by chronic sc chronic nicotine treatments : dependance on presynaptic i.

Xavier Vigé and Mike Briley

Departement de Pharmacologie Biochimique.
Centre de Recherche Pierre Fabre
17 Ave. Jean Moulin, 81100 Castres, France.

Many authors have reported that chronic scopolamine treatment increases muscarinic receptor density in the central nervous system [see for example 1]. We have recently shown [3] that chronic treatment with the selective muscarinic antagonist, scopolamine, (10 mg/kg i.p.) for 21 days also induces a significant up-regulation in the nicotinic receptor population. In the frontoparietal cortex of treated rats we observed an increase in the density of nicotinic receptors of 12-20 % as measured by the specific binding of [^{3}H](-)nicotine. It has also been demonstrated that up-regulation of nicotinic receptors may also be induced by chronic administration of the agonist, nicotine [2].

In this study, we have compared the effect of both chronic scopolamine and nicotine treatments in rats lesioned with ibotenic acid at the level of the nucleus basalis of Meynert, the area which innervates the frontoparietal cortex. The results show a loss of scopolamine-induced up-regulation in lesioned rats having decreases of choline acetyltransferase activity of greater than 35 %. In contrast the up-regulation caused by chronic nicotine (1 mg/kg s.c. twice daily for 10 days) was not abolished by the lesion.

These results suggest that blockade of (presynaptic ?) muscarinic receptors by scopolamine increases the firing of the cholinergic neurones which would increase the level of acetylcholine at the synaptic level and hence the activation of the nicotinic receptor. It is consistent with this hypothesis that the effect of scopolamine would be abolished by cholinergic denervation whereas the effect of the direct acting agonist, nicotine, would not be affected.

Scopolamine treatment ; nicotinic receptor ; [^{3}H](-)Nicotine ; nucleus basalis of Meynert ; Ibotenic acid.

1 - Russell R.W., Ehlert F.J. and Hwa J.J., (1986) Psychopharmacol. 88, 33-39.
2 - Schwartz R.D. and Kellar K.J., (1985) J. Neurochem. 45, 427-433.
3 - Vige X. and Briley M., (1988) Neurosci. Letters (In press).